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THE RELATIVE THERAPEUTIC VALUE OF ANTITOXIC GLOBULIN SOLUTION AND THE WHOLE SERUM FROM WHICH IT WAS DERIVED.*

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THE process of concentrating diphtheria antitoxin, devised in this laboratory by Gibson,[1] has been adopted by a large number of American manufacturers, and has resulted in the extensive employment of the product in this country. Careful clinical trials have convinced us that this antitoxic globulin preparation possesses all the therapeutic properties of the native serum.

Cruveilhier[2] has recently stated that the customary method of standardizing antidiphtheric serum is not comprehensive enough, in that it leaves out of account therapeutic serum-constituents other than the antitoxin. While we believe that the work of Steinhardt and Banzhaf in the preceding paper[3] has effectually disposed of this objection, it seemed advisable to study the question in connection with the Gibson process of concentration, and with the fractional precipitation of the antiserum according to the method of Banzhaf and Gibson.[4]

Our general method of procedure was similar to that outlined in the preceding paper. In this instance, we mixed antitoxic citrated plasma from several horses and then tested this mixture before and after eliminating the non-antitoxic proteins. We also compared the different globulin fractions of the plasma.

In these experiments, the following mixtures of citrated plasma were used:

Mixture 1.—Fifty liters of citrated plasma were obtained from five horses as follows:

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[1] *Jour. Biol. Chem.*, 1, p. 161.

[2] *Ann. de l'Inst. Past.*, 1904, 18, p. 249.

[3] *Jour. Infect. Dis.*, 1908, 5, p. 203.

[4] *Jour. Biol. Chem.*, 3, p. 253.

1,000 c.c. from horse 262, part of one bleeding.
3,600 c.c. " " 305, " " two bleedings.
5,400 c.c. " " 306, " " three bleedings.
1,500 c.c. " " 310, " " one bleeding.
38,500 c.c. " " 311, full six bleedings.

The potency of this mixture, at the time of these experiments, was 700 units per c. c.

Mixture 2.—Forty liters of citrated plasma were obtained from three horses as follows:

8,000 c.c. from horse 288, full bleeding.
6,100 c.c. " " 305, full bleeding.
25,900 c.c. " " 299, full four bleedings.

The original potency of this mixture was 475 units per c.c. At the time of these experiments, however, the potency had dropped to 450 units per c.c.

(Mixture 1.) The 50 liters were refined and concentrated by Gibson's method, to 16 liters of antitoxic globulin solution containing 1,650 units per c.c.

Gibson's method briefly is as follows: The diluted citrated plasma is precipitated with an equal amount of saturated ammonium sulphate solution and the antitoxic proteins are separated by extracting the precipitate with saturated sodium chloride solution. The soluble antitoxic proteins are then reprecipitated from the saturated sodium chloride solution with acetic acid. This filtered precipitate is then partially dried between filter papers and dialyzed in parchment in running water.

(Mixture 2.) The 40 liters were divided into two lots. One lot of 20 liters was diluted with an equal amount of water and refined and concentrated by Gibson's method. The final product, designated 77A, amounted to 5,200 c.c., each c.c. containing 1,450 units per c.c. At the time of these experiments, however, the potency had dropped to 1,375 units per c.c.

The second lot of 20 liters was diluted with an equal amount of water and the antitoxic globulins obtained by three fractional precipitations at concentrations corresponding to 3.3 c.c., 3.3–3.8 c.c., and 3.8–5.0 c.c. of saturated ammonium sulphate in 10 c.c.[1]

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[1] There exists at the present time considerable confusion in comprehending the methods and basic principles of ammonium sulphate fractional precipitation of proteins. The nomenclature which we have employed and which designates the number of c.c. of saturated ammonium sulphate solution in 10 c.c.

The soluble antitoxic proteins in saturated sodium chloride solution were prepared in the usual way. The antitoxic globulin solution from the first fraction, designated 77B, amounted to 1,440 c.c., each c.c. containing 1,150 units per c.c. At the time of these experiments, however, the potency had dropped to 1,025 units per c.c. The second fraction, designated 77C, amounted to 1,400 c.c., each c.c. containing 1,350 units per c.c. At the time of these experiments, however, its potency had dropped to 1,175 units per c.c. The third fraction, designated 77D, amounted to 2,050 c.c., each c.c. containing 1,750 units per c.c. At the time of these experiments, however, the potency had dropped to 1,550 units per c.c.

The animals used in these experiments were active, healthy, normal guinea-pigs weighing between 250–260 grams.

The three diphtheria strains were the same as in the preceding paper.

PREVENTIVE EXPERIMENTS.

Mixture 1.

A uniform sample of the mixture of citrated plasma containing 700 units per c.c., was compared with the finished product of antitoxic globulin solution containing 1,650 units per c.c.

Experiment 1.—Six guinea-pigs were inoculated subcutaneously with 1, $\frac{3}{4}$, $\frac{1}{2}$, $\frac{1}{4}$, $\frac{1}{8}$, and $\frac{1}{16}$-unit of the 700-unit citrated plasma, respectively; a parallel set of six guinea-pigs received the same antitoxic unit values of the 1,650 antitoxic globulin solution. Twenty-four hours later, all the animals, as well as a control guinea-pig, received a fatal dose of culture No. 1.[1] The control animal died in 27 hours. The $\frac{3}{4}$-unit of both the citrated plasma and the antitoxic globulin solution protected.

Experiment 2.—In this, culture No. 2[1] was used in place of culture No. 1; otherwise it was parallel with the first experiment. The results were: The control animal died between 33 and 36 hours.

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of the *precipitated* mixture has been used by some authorities; it avoids the confusion developed by the use of such terms as "per cent $(NH_4)_2SO_4$ solution," "per cent saturation $(NH_4)_2SO_4$," "per cent of saturated $(NH_4)_2SO_4$ solution," and "per cent saturation $(NH_4)_2SO_4$ solution," and it seems the simplest and best *practical* expression of degrees of saturation yet suggested. We advise that this method be employed in future papers on fractional precipitation.

[1] Culture Nos. 1 and 2: No. 1, a moderate toxin producer; No. 2, a weak toxin producer. Both of these were freshly isolated from the throats of diphtheria patients at the Department of Health Hospital.

The $\frac{1}{4}$-unit of both the citrated plasma and the antitoxic globulin solution protected.

Experiment 3.—In this, culture No. 8[1] was used in place of culture No. 2; otherwise it was parallel with the first and second experiments. The results were: The control animal died in 28 hours. The $\frac{1}{2}$-unit of both the citrated plasma and the antitoxic globulin solution protected.

TABLE 1.
PREVENTIVE EXPERIMENTS.

AMOUNT INJECTED IN UNITS	EXPERIMENT 1 WITH CULTURE NO. 1		EXPERIMENT 4 WITH CULTURE NO. 1				
	Sample of Plasma 700 Units per c.c.	Concentrated Antitoxic Glob. Sol. 1,650 Units per c.c.	Sample of Plasma 450 Units per c.c.	Concentrated Antitoxic Glob. Sol. 77A 1,375 Units per c.c.	First Fract. Antitoxic Glob. Sol. 77B 1,025 Units per c.c.	Second Fract. Antitoxic Glob. Sol. 77C 1,175 Units per c.c.	Third Fract. Antitoxic Glob. Sol. 77D 1,550 Units per c.c.
1 unit	lived	lived	lived	lived	lived	lived	lived
$\frac{3}{4}$ "	lived	lived	lived	lived	lived	lived	lived
$\frac{1}{2}$ "	died	died	lived	lived	lived	lived	lived
$\frac{1}{4}$ "	died	died	died	died	died	died	died
$\frac{1}{8}$ "	died	died	died	died	died	died	died
$\frac{1}{16}$ "	died	died	died	died	died	died	died
	Control died in 27 hrs.		Controls died in $28\frac{1}{2}$ and 29 hrs.				
	EXPERIMENT 2 WITH CULTURE NO. 2		EXPERIMENT 5 WITH CULTURE NO. 2				
1 unit	lived	lived	lived	lived	lived	lived	lived
$\frac{3}{4}$ "	lived	lived	lived	lived	lived	lived	lived
$\frac{1}{2}$ "	lived	lived	lived	lived	lived	lived	lived
$\frac{1}{4}$ "	lived	lived	died	died	died	died	died
$\frac{1}{8}$ "	died	died	died	died	died	died	died
$\frac{1}{16}$ "	died	died	died	died	died	died	died
	Control died in 33–36 hrs.		Controls died in $28\frac{1}{2}$ hrs.				
	EXPERIMENT 3 WITH CULTURE NO. 8		EXPERIMENT 6 WITH CULTURE NO. 8				
1 unit	lived	lived	lived	lived	lived	lived	lived
$\frac{3}{4}$ "	lived	lived	lived	lived	lived	lived	lived
$\frac{1}{2}$ "	lived	lived	lived	lived	lived	lived	lived
$\frac{1}{4}$ "	died	died	lived	lived	lived	lived	lived
$\frac{1}{8}$ "	died	died	died	died	died	died	died
$\frac{1}{16}$ "	died	died	died	died	died	died	died
	Control died in 28 hrs.		Controls died in 30–32 hrs.				

Mixture 2.

In Experiments 4, 5, and 6, we instituted the following comparisons:

a) Between a sample of citrated plasma (Mixture 2) containing

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[1] Park and Williams, culture No. 8. This is a strong toxin producer and has been the stock in this laboratory for 12 years.

450 units per c.c., and the finished product of antitoxic globulin solution containing 1,375 units per c.c.

b) Between the same citrated plasma (Mixture 2) and the three fractions of the antitoxic globulins; of these Fraction 1 contained 1,025 units per c.c; Fraction 2 contained 1,175 units per c.c.; and Fraction 3, 1,550 units per c.c.

Experiment 4.—This required 30 guinea-pigs as follows: Six guinea-pigs received 1, $\frac{3}{4}$, $\frac{1}{2}$, $\frac{1}{4}$, $\frac{1}{8}$, and $\frac{1}{16}$-unit of the 450-unit citrated plasma, respectively; six received the same antitoxic unit values of the 1,375-unit antitoxic globulin solution, 77A; six received the same antitoxic values of the 1,025-unit, first fraction, antitoxic globulin solution; six received the same antitoxic values of the 1,175-unit, second fraction, antitoxic globulin solution; six received the same values of the 1,550-unit, third fraction, antitoxic globulin solution. Twenty-four hours later, all the animals, as well as two control guinea-pigs, received a fatal dose of culture No. 1. The results of the five lots were uniform. The control animals died between 28½ and 29 hours. The ½-unit of the citrated plasma, the antitoxic globulin solution, and the fractions of the antitoxic globulins protected.

Experiment 5.—In this, culture No. 2 was used in place of culture No. 1; otherwise it was parallel with experiment 4. The results of the five lots were uniform. The control animals died in 28½ hours. The ½-unit of the citrated plasma, the antitoxic globulin solution, and the fractions of the antitoxic globulins protected.

Experiment 6.—In this, culture No. 8 was used in place of culture No. 2; otherwise it was parallel with experiments 4 and 5. The results of the five lots were uniform. The control animals died between 30 and 32 hours. The ¼-unit of the citrated plasma, the antitoxic globulin solution, and the fractions of the antitoxic globulins protected.

CURATIVE EXPERIMENTS.

Mixture 1.

The guinea-pigs were inoculated subcutaneously with a fatal dose of culture No. 1, and divided into two lots. At intervals of two hours trom the fourth to the eighth hour, each animal of the first lot received subcutaneously 25 units of the 700-unit citrated plasma;

and each animal of the second lot received 25 units of the 1,650-unit antitoxic globulin solution. The results were: The control animal died in 27 hours. The animals receiving the curative dose after four and six hours, lived; while those receiving the curative dose after eight hours, died.

TABLE 2.

CURATIVE EXPERIMENTS.

No. of Hours after Injection of Culture	Experiment 1 with Culture No. 1		Experiment 4 with Culture No. 1				
	Sample of Plasma 700 Units per c.c. (25 Units Injected)	Concentrated Antitoxic Glob. Sol. 1,650 Units per c.c. (25 Units Injected)	Sample of Plasma 450 Units per c.c. (25 Units Injected)	Concentrated Antitoxic Glob. Sol. 77A 1,375 Units per c.c. (25 Units Injected)	First Fract. Antitoxic Glob. Sol. 77B 1,025 Units per c.c. (25 Units Injected)	Second Fract. Antitoxic Glob. Sol. 77C 1,175 Units per c.c. (25 Units Injected)	Third Fract. Antitoxic Glob. Sol. 77D 1,550 Units per c.c. (25 Units Injected)
4 hours	lived	lived	lived	lived	lived	lived	lived
6 "	lived	lived	lived	lived	lived	lived	lived
8 "	died	died	died	died	died	died	died
	Control died in 27 hrs.			Controls died in 28 hrs.			
	Experiment 2 with Culture No. 2		Experiment 5 with Culture No. 2				
4 hours	lived	lived	lived	lived	lived	lived	lived
6 "	lived	lived	lived	lived	lived	lived	lived
8 "	died	died	died	died	died	died	died
	Control died in 26½ hrs.			Controls died in 27–27½ hrs.			
	Experiment 3 with Culture No. 8		Experiment 6 with Culture No. 8 (in Duplicate)				
4 hours	lived	lived	2 lived	2 lived	2 lived	2 lived	2 lived
6 "	died	died	2 died	2 died	2 died	2 died	2 died
8 "	died	died	2 died	2 died	2 died	2 died	2 died
	Control died in 25½ hrs.			Controls died in 25 hrs.			

Experiment 2.—In this, culture No. 2 was used in place of culture No. 1; otherwise it was parallel with the first experiment. The results were: The control animal died in 26½ hours. The animals receiving the curative dose after four and six hours, lived; while those receiving the curative dose after eight hours, died.

Experiment 3.—In this, culture No. 8 was used in place of culture No. 2; otherwise it was parallel with experiments 1 and 2. The results were: The control animal died in 25½ hours. The animals receiving the curative dose after four hours, lived; after six and eight hours, died.

Mixture 2.

Experiment 4.—The guinea-pigs received subcutaneously a fatal dose of culture No. 1, and were divided into five lots. At intervals of two hours from the fourth to the eighth hour, each animal of the first lot received subcutaneously 25 units of the 450-unit citrated plasma; each animal of the second lot 25 units of the 1,375-unit antitoxic globulin solution, 77A; the third lot 25 units of the 1,025-unit, first fraction, antitoxic globulin solution; the fourth lot 25 units of the 1,175-unit, second fraction, antitoxic globulin solution; the fifth lot 25 units of the 1,550-unit, third fraction, antitoxic globulin solution. The results of the five lots were uniform. The control animals died in 28 hours. All the animals receiving the curative dose after four and six hours, lived; after eight hours, died.

Experiment 5.—In this, culture No. 2 was used in place of culture No. 1; otherwise it was parallel with experiment 4. The results of the five lots were again uniform. The control animals died in 27 and 27½ hours. All the animals receiving the curative dose after four and six hours, lived; after eight hours, died.

Experiment 6.—In this, which was in duplicate, culture No. 8 was used, in place of culture No. 2; otherwise it was parallel with experiments 4 and 5. The duplicate results of the five lots were again uniform. The control animals died in 25 hours. All the animals receiving the curative dose after four hours, lived; after six and eight hours, died.

In view of the results obtained in these experiments and in those of Steinhardt and Banzhaf in the preceding paper, it is obvious that the therapeutic value of the plasma is not appreciably impaired through the process of eliminating the albumins and other non-antitoxic proteins by the salting out methods employed, and the final dialyzation of the concentrated product.